Tendon-derived ECM thermoresponsive hydrogel to deliver iTenocytes for scarless tendon healing

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INTRODUCTION: Cell-based therapies for tendon and ligament healing hold significant promise, but substantial challenges remain. When delivered via direct injection, cells often exhibit rapid loss of viability and are easily displaced from the defect site. To be effective, such therapies therefore require a supportive delivery system that enhances cell retention, survival, and local integration. To address this, we built a tendon-derived, thermoresponsive ECM hydrogel (TG) for minimally invasive delivery of SCX overexpressing iMSCs (Tenocytes). The TG is expected to protect cells during application and conform to defect zone in tendon while steering tenogenic maturation. Moreover, pharmacologically inducing mechanosensitivity pathways mainly through PIEZO1 activation may activate tenogenic program in iTenocytes and allow mature tendon-regenerating cells to be delivered at the tendon defect zone non-invasively through loading in injectable TG **(Fig. 1A)**.

METHODS: TG was produced by chemical decellularization of porcine Achilles tendon and pepsin digestion **(Fig. 1B).** The iTenocytes were produced via lentiviral transduction to achieve stable expression of SCX in induced mesenchymal stem cells (iMSCs). The TG was compared to PureCol (PC, Advanced Biomatrix) at the protein level. The iTenocytes were grown on TG for 7 days to assess morphology and whole-transcriptome changes, with bulk RNA sequencing (RNA-seq) compared to PC. To test PIEZO1-driven mechanosensitivity in tenogenesis, we applied Yoda1 at different concentrations and assessed gene expression of mechanoresponsive and tenogenic genes. The activation was benchmarked against PIEZO1 inhibition using Gsmtx4.

RESULTS: Porcine tendons were successfully decellularized and a strain-thinning biocompatible and injectable TG was produced **(Fig. 1C, D).** The TG demonstrated drastically different protein content with higher collagen type 1 (COL1) presence **(Fig. 1E)** and proteins taking part in cytoskeletal and ECM interactions **(Fig. 1F)** comparing to PC. TG supported robust iTenocyte proliferation and migration into the environment **(Fig. 2A)**, enabling effective colonization. RNA-seq analysis revealed significant upregulation of tenogenic markers when cultured for 7 days **(Fig. 2B)**. Moreover, mechanosensitive and tendon ECM–related markers were increased in TG-cultured iTenocytes compared to PC **(Fig. 2C)**. Furthermore, iTenocytes were evaluated for their responsiveness to pharmacological mechanostimulation. Within 7 days, iTenocytes exhibited pronounced alignment and significant proliferation on 2D substrates **(Fig. 2D)**. PIEZO1 expression increased in a dose-dependent manner with YODA1 treatment, ranging from 0.25 to 100µM **(Fig. 2E)**. Notably, a threshold response was observed at 4 µM, where PIEZO1 activation coincided with loss of COL1A1 expression. Furthermore, mechanosensitive tenogenic markers, including Mohawk (MKX) and Early Growth Response-1 (EGR1), were markedly upregulated, indicating a robust tenogenic response **(Fig. 2F)**.

DISCUSSION: Given the low abundance of resident tendon progenitors, functional repair requires the introduction of highly proliferative and capable cells to restore structure and prevent scarring. Here, we successfully developed a tenogenic, injectable, and bioresorbable gel that can be delivered with iTenocytes in a minimally invasive manner to induce a regenerative response. In addition, we aimed to mature iTenocytes prior to loading into TG, thereby priming them to effectively migrate into the host tissue, build ECM, and populate the defect zone to promote functional tendon regeneration. Current work is evaluating the host response to TG/iTenocytes after PIEZO1 stimulation in a partial Achilles tendon defect model in Sprague-Dawley rats to determine iTenocyte retention and viability at the tendon defect site.

SIGNIFICANCE: Effective implantation, survival, and regenerative function of transplanted cells has remained a long-standing challenge in tendon cell therapy. This work demonstrates the use of a tendon-mimicking, thermoresponsive gel to support pharmacologically preconditioned cells via mechanosensitive pathways, enabling the delivery of mature, functionally primed cells.

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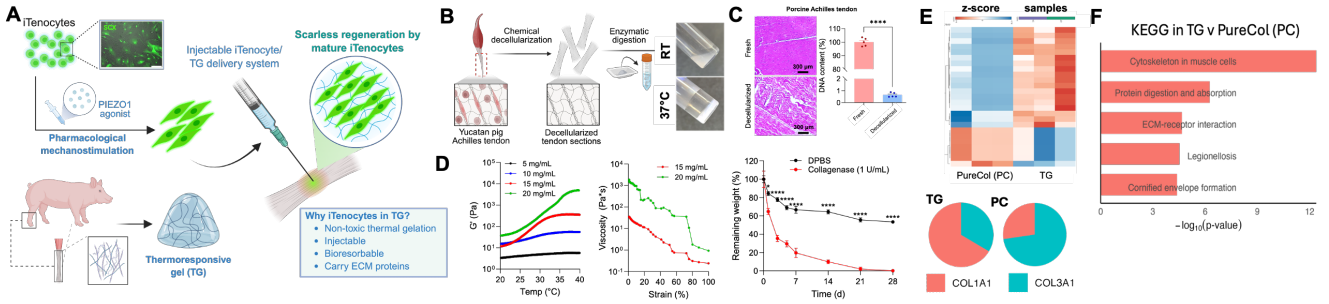

Figure 1. A) GFP-tagged SCX overexpressing iTenocytes have been investigated for pharmacological mechanostimulation using PIEZO1 agonist YODA1. Our work aims to load primed iTenocytes in TG, where they will be non-invasively transplanted in tendon. **B)** TG can be gelled at 37±2°C. **C)** Successful decellularization was confirmed by complete cell removal and residual DNA levels (<50 ng/mg dry tissue). **D)** The rheological and degradation analyses showed that TG is an injectable and bioresorbable gel. **E)** Proteomic comparison of TG and PC revealed distinct matrix profiles. TG exhibited a higher COL1/COL3 ratio, whereas PC showed the opposite trend. **F)** KEGG analysis demonstrated that TG upregulated cytoskeletal and ECM-associated pathways.

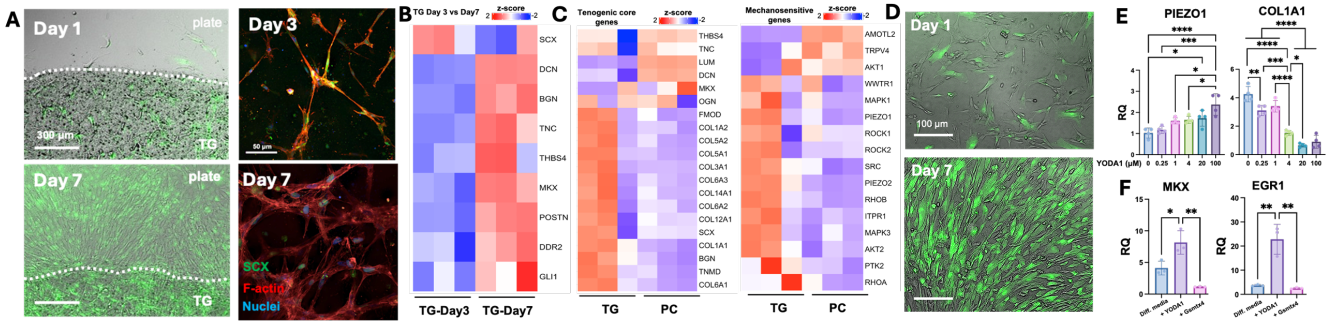

Figure 2. A) iTenocytes proliferated within TG and invaded the surrounding microenvironment, showing interconnected expansion and successful colonization. TG not only enhanced proliferation but also markedly upregulated tenogenic gene expression. **B)** Compared to PC, TG more effectively induced a tenogenic response and additionally activated mechanosensitive gene expression. **C)** On 2D tissue culture plates, iTenocytes treated with the PIEZO1 agonist YODA1 at 4 µM exhibited proliferation and alignment after 7 days. **D)** Dose–response analysis at the concentrations of 0.25 to 100 µM showed increased PIEZO1 expression without cytotoxicity. A threshold effect was observed at 4 µM, where PIEZO1 activation coincided with loss of COL1A1 expression. Furthermore, early tenogenic markers MKX and EGR1 were markedly upregulated, underscoring the potential of mechanosensitive pathways to drive iTenocyte maturation.